

2

END FILE COPY

AD _____

CONTRACT NO: DAMD17-88-C-8069

AD-A228 158

TITLE: DEVELOPMENT OF AN ASSAY TO DETECT ANTIBODIES TO HIV-2
USING RECOMBINANT DNA DERIVED ANTIGENS

PRINCIPAL INVESTIGATOR: Gerald A. Beltz, Ph.D.

CONTRACTING ORGANIZATION: Cambridge BioScience Corporation
365 Plantation Street
Worcester, MA 01605

REPORT DATE: September 11, 1990

TYPE OF REPORT: Final Report

PREPARED FOR: U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
Fort Detrick, Frederick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for public release;
distribution unlimited

The findings in this report are not to be construed as an official Department of the Army position unless so designated by other authorized documents.

DTIC
ELECTE
OCT 16 1990
D
E

REPORT DOCUMENTATION PAGE				Form Approved OMB No. 0704-0188	
1a. REPORT SECURITY CLASSIFICATION Unclassified			1b. RESTRICTIVE MARKINGS		
2a. SECURITY CLASSIFICATION AUTHORITY			3. DISTRIBUTION / AVAILABILITY OF REPORT Approved for public release; distribution unlimited		
2b. DECLASSIFICATION / DOWNGRADING SCHEDULE					
4. PERFORMING ORGANIZATION REPORT NUMBER(S)			5. MONITORING ORGANIZATION REPORT NUMBER(S)		
6a. NAME OF PERFORMING ORGANIZATION Cambridge BioScience Corporation		6b. OFFICE SYMBOL (if applicable)	7a. NAME OF MONITORING ORGANIZATION		
6c. ADDRESS (City, State, and ZIP Code) 365 Plantation Street Worcester, MA 01605			7b. ADDRESS (City, State, and ZIP Code)		
8a. NAME OF FUNDING / SPONSORING ORGANIZATION U.S. Army Medical Research & Development Command		8b. OFFICE SYMBOL (if applicable)	9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER Contract No. DAMD17-88-C-8069		
8c. ADDRESS (City, State, and ZIP Code) Fort Detrick Frederick, Maryland 21702-5012			10. SOURCE OF FUNDING NUMBERS		
		PROGRAM ELEMENT NO. 63105A	PROJECT NO. 3M2 63105DH29	TASK NO. AB	WORK UNIT ACCESSION NO. 064
11. TITLE (Include Security Classification) Development of an assay to detect antibodies to HIV-2 using recombinant DNA derived antigens.					
12. PERSONAL AUTHOR(S) Gerald Beltz					
13a. TYPE OF REPORT Final Report		13b. TIME COVERED FROM 6/30/88 TO 3/31/90		14. DATE OF REPORT (Year, Month, Day) September 11, 1990	
15. PAGE COUNT 27					
16. SUPPLEMENTARY NOTATION					
17. COSATI CODES			18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)		
FIELD	GROUP	SUB-GROUP	RA I; HTLV-III; Recombinant; DNA; Antigens; ELISA; Antibody; Screening; HIV-2		
06	03				
06	13				
19. ABSTRACT (Continue on reverse if necessary and identify by block number)					
<p>To develop an EIA capable of detecting HIV-2 seropositive individuals, we cloned and expressed contiguous regions of the HIV-2 envelope gene. One region that we call K1, when expressed in E. coli, produces a 36 KD protein that reacts with all seropositive samples tested, indicating that it (K1) contains the immunodominant epitopes of the envelope protein. Other regions show little or no reactivity with seropositive samples. We have developed procedures for large scale purification of the K1 protein and have used the purified material to produce a highly sensitive screening assay for the detection of HIV-2 seropositive individuals.</p>					
20. DISTRIBUTION / AVAILABILITY OF ABSTRACT <input type="checkbox"/> UNCLASSIFIED/UNLIMITED <input type="checkbox"/> SAME AS RPT <input type="checkbox"/> DTIC USERS			21. ABSTRACT SECURITY CLASSIFICATION Unclassified		
22a. NAME OF RESPONSIBLE INDIVIDUAL Mrs. Virginia M. Miller			22b. TELEPHONE (Include Area Code) (301) 663-7325		22c. OFFICE SYMBOL SGRD-RMI-S

FOREWORD

Opinions, interpretations, conclusions and recommendations are those of the author and are not necessarily endorsed by the U.S. Army.

Where copyrighted material is quoted, permission has been obtained to use such material.

Where material from documents designated for limited distribution is quoted, permission has been obtained to use the material.

141 Citations of commercial organizations and trade names in this report do not constitute an official Department of the Army endorsement or approval of the products or services of these organizations.

In conducting research using animals, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (NIH Publication No. 86-23, Revised 1985).

For the protection of human subjects, the investigator(s) have adhered to policies of applicable Federal Law 45CFR46.

141 In conducting research utilizing recombinant DNA technology, the investigator(s) adhered to current guidelines promulgated by the National Institutes of Health.

PI Signature

Date

Accession For	
NTIS GRA&I	<input checked="checked" type="checkbox"/>
DTIC TAB	<input checked="checked" type="checkbox"/>
Unannounced	<input type="checkbox"/>
Justification	
By	
Distribution/	
Availability Codes	
Dist	Avail and/or Special

A-1



TABLE OF CONTENTS

INTRODUCTION.....	2
MATERIALS AND METHODS.....	3
RESULTS.....	5
SUMMARY.....	15
REFERENCES.....	17
FIGURES AND TABLES.....	18

INTRODUCTION

HIV-2 was initially identified in 2 patients from Western Africa who developed AIDS-like symptoms but were seronegative for HIV-1 by viral lysate immunoassays (1). The HIV-2 virus cultured from PBL's of these patients was morphologically similar to HIV-1 and exhibited similar cell tropism for CD4+ cells. Preliminary serological studies indicated that while HIV-2 was related to HIV-1, it was more closely aligned with SIV_{mac}.

Subsequent cloning and sequencing of HIV-2 confirmed these results (2,3). While the overall genetic organization of HIV-2 is similar to HIV-1, there is considerable sequence diversity between the two. The degree of sequence divergence varies between coding regions. While corresponding gag and pol sequences show 50-60% homology at the amino acid level, the envelope gene shows less sequence conservation, only 30-40%. The lower degree of homology extends to the short open reading frames, rev and sor (2,3).

HIV-2 infected individuals have been identified in Africa, Europe, North and South America (4,5) and epidemiological studies show that HIV-2 is associated with AIDS in Western Africa (6,7). Since HIV-2 exhibits limited cross reactivity with HIV-1, it is important to develop an assay capable of detecting HIV-2.

This report describes the development of an HIV-2 ELISA

using recombinant DNA derived antigens expressed in E. coli.

Recombinant derived antigens offer several advantages in immunoassays. Thorn et al. (12) have developed a protein CBre3, for use in an HIV-1 EIA. The protein, also expressed in E. coli, contains the major immunodominant epitopes of the HIV-1 envelope protein. Because it is expressed at high levels, large amounts of the protein can be used in individual assays, and the protein can be purified free of contaminants. A CBre3 EIA is sensitive enough to detect envelope antikodies in seropositive patients before the antibodies are detected in a viral lysate Western blot (12).

Our strategy was to identify immunodominant regions of the HIV-2 envelope protein by (i) cloning and expressing in E. coli non-overlapping subfragments of the HIV-2 envelope protein and (ii) testing the expressed proteins for reactivity with sera from HIV-2 seropositive individuals.

By cloning regions of the HIV-2 envelope gene, we have identified and expressed a protein called K1, that contains the major immunodominant epitopes of the HIV-2 envelope gene.

We have developed a purification protocol for K1 and used the purified protein in an HIV-2 ELISA. Our results indicate that K1 can detect all HIV-2 seropositive samples that we have tested.

MATERIALS AND METHODS

HIV-2 Envelope Gene.

The initial plasmid, K3D, used for cloning, was kindly

provided by Dr. Genoveffa Franchini (8). The plasmid consists of a 2.7 KB KpnI fragment containing the entire HIV-2 envelope gene, cloned into SP65. The 2.7 KB fragment was derived from an infectious HIV-2 clone, HIV-2_{SBL/ISY}, isolated from a genomic library of HUT 78 cells infected with the HIV-2 strain SBL6669. The restriction map of the infectious clone, and the location of the 2.7 KB KpnI fragment is shown in Figure 1.

Cloning and Expression of the Envelope Genes.

The precise location of the subgenomic envelope proteins are described in the results section. DNA fragments coding for K13 and K3 were generated by Sau3AI digestion of K3D DNA. The appropriate fragments were isolated from agarose gels by electroelution and cloned into the Bam HI site of the expression vector pCBC (see below). K18 was obtained by digesting K3D with PvuII, adding Bam HI linkers and digesting with Sau3AI. The fragment was cloned into pCBC. Cloning of the K1 coding fragment was done by digesting K3D with EcoRI and treating with Klenow polymerase to fill in the ends. BglII linkers were added to the fragment and the K1 coding fragment was isolated following digestion with Sau3AI. K2 was cloned by digesting K3D with StuI, adding Bam HI linkers and digesting with Sau3AI. K1 and K2 coding fragments were also inserted into the expression vector pCBC. All DNA manipulations were according to Maniatis (10).

The E. coli expression vector was pCBC. The vector, similar to pJL6 described by Lautenberger et al. (9) contains the bacteriophage lambda pL promotor, synthetic ribosome binding

site, and the first 13 amino acids of the lambda CII gene. DNA fragments are cloned into a Bam HI site distal to the CII coding region resulting in a fusion protein containing a 13 amino acid leader of CII.

Envelope constructs in pCBC were used to transform E. coli MZ-1. The MZ-1 strain contains a temperature sensitive lambda C₁₈₅₇ gene (9). At 32°C the repressor binds to the pL promotor inhibiting transcription. Induction by temperature shift from 32°C to 42°C allows transcription of pL distal genes. MZ-1 cells are grown at 32°C to OD₅₅₀ of 0.500-1.000 and then induced at 42°C for one hour.

Analysis of Envelope Proteins

Proteins from uninduced and induced E. coli were electrophoresed in polyacrylamide gels and analyzed by Coomassie blue staining and Western blotting (10). Blots were blocked in 1 X TBS, 0.1% Brij for 60' and incubated for 60' with sera diluted 1:100 in blocking buffer. Blots were washed 3 X with 1 X TBS and incubated with HRP conjugated goat anti-human antibody for 60'. Blots were washed as above and then incubated with the HRP substrate 4 Chloro/Napthol.

Purification of K1 Protein and R1A Development

Purification of the recombinant protein and development of an HIV-2 EIA are described in text.

RESULTS

The starting material for cloning was an HIV-2 subgenomic fragment containing the complete HIV-2 envelope gene. The

fragment, kindly provided by Dr. Genoveffa Franchini, was isolated from a genomic HIV-2 clone and is shown in Figure 1.

Figure 2 shows a hydropathy plot of the HIV-2 envelope protein. The regions represented beneath the plot, K18, K13, K3, K1 and K2 represent the individual subfragments that were cloned and expressed.

The first clone that we tested was K1. Based on our work with HIV-1, we predicted that this region of the viral genome would code for an immunodominant envelope antigen. E. coli strain MZ-1, containing a thermolabile cI repressor, was transformed with a plasmid containing the K1 region of the HIV-2 envelope gene downstream of the lambda pL promotor. As described, proteins from uninduced and induced E. coli containing the K1 coding fragment were analyzed.

Figure 3 shows a picture of a Coomassie stained acrylamide gel. The samples in each lane are as follows. Lane 1 has prestained molecular weight markers. Lanes 2 and 3 are uninduced and induced extracts of bacteria expressing the comparable region from the envelope gene of Simian Immunodeficiency Virus (SIV). Prior to beginning work on the HIV-2 clone, we had expressed a protein from the envelope region of SIV containing SIV env amino acids 365 to 646, that reacted strongly with HIV-2 samples. This SIV protein is Acc1. Acc1 is 80% homologous in amino acid sequence to the comparable HIV-2 protein. Since it had been cloned and expressed, and had been shown to be useful in detecting HIV-2 seropositive patients, it served as a positive

control in the assays. The pairs of lanes, 4,5; 6,7; 8,9; 10,11; and 12,13, are uninduced and induced cultures (respectively) of 5 independently isolated clones all expressing the recombinant HIV-2 K1 protein. The arrow identifies the protein present in the induced, but not in the uninduced, cultures of the HIV-2 recombinants.

Western blot analysis was used to show that the induced protein is coded for by the HIV-2 DNA. A duplicate gel, as shown in Figure 3, was blotted and reacted with antibody raised in rabbits against the SIV envelope protein Acc1. Since Acc1 and the HIV-2 envelope protein K1 are approximately 80% homologous, we predicted that polyclonal antisera raised against Acc1 should react with K1. Figure 4 shows the Western blot. The band in lane 3 represents anti-Acc1 antibody reacting with the Acc1 protein. Reactivity in lanes 5, 7, 9, 11 and 13 represent antibody recognizing the induced HIV-2 protein, K1. The stronger reactivity of anti-Acc1 antibody to K1 reflects the higher expression level of the HIV-2 protein. We estimate that expression of the K1 protein represents at least 5% of the total protein.

Reactivity of K1 with Human Sera

Western blots of proteins from K1 expressing cultures (comparable to material shown in Figure 2 and 3) were used to assay serum samples from HIV-2 infected individuals collected in West Africa, where there is a high incidence of HIV-2. Samples were screened by commercial EIAs and provided by Dr. M'Boup of

the University of Senegal. Figure 5 shows the Western results from 47 different serum samples. The majority of samples react strongly with the K1 protein. The strips which are unreactive by Western blot (3, 18, and 44), are negative by viral lysate Western blots (Genetic Systems) and by radioimmune precipitations (data not shown). We tested a second set of seropositive samples by Western blot with comparable results (data not shown). In all, we have tested 80 West African samples by Western blot for reactivity to the subunit HIV-2 K1 protein. Our results continue to show that the K1 protein detects all HIV-2 seropositive samples.

Reactivity of K3, K18, K13, and K2 with HIV-2 Seropositive

Recombinant HIV-2 envelope proteins from 4 additional clones were also analyzed for reactivity with sera from seropositive individuals. All constructs were cloned into the expression vector pCBC. Inductions were done as described for the K1 protein. The K3 protein is slightly larger than K1 because it contains an additional 64 amino acids at the N-terminus. Reactivity of sera to both K3 and K1 was compared. In terms of specificity, sensitivity, and signal observed with positive samples, there is no significant difference between the two proteins.

The other HIV-2 envelope proteins that we have expressed are as follows (see Figure 2):

K18. A 15 KD protein that begins at amino acid 7 and ends at amino acid 135. The Pvu II site at amino acid 7 was converted

to a Bam HI site. The 3' end of the DNA fragment is a Sau 3AI site which is compatible with the Bam HI site in the vector.

K13. The amino terminus of K13 begins at amino acid 136 which is just distal to the carboxy terminus of the K18 clone. The 3' end of the gene is at amino acid 320 which is also a Bam HI compatible Sau 3AI site. K13 is expected to code for a protein of 18 KD.

K2. This is a small protein coded for by sequences just proximal to the second major hydrophobic domain of HIV-2 (figure 2). The protein extends from amino acid 700-797.

Western blots were made for each of the proteins, K18, K13 and K2 and the blots were reacted with HIV-2 seropositive samples. The results are shown in Table I. All three of the constructs react with only a minority of the samples or with none at all.

Because of the low reactivity of the proteins with sera that had been shown to react strongly with the K1 protein, we tested the K18, K13, and K2 Western blots to ensure that the proteins were expressed and transferred during blotting. Since all three proteins contain a 13 amino acid leader sequence coded for by the CII gene of lambda, the Western blots containing these proteins were reacted with antibodies previously shown to recognize the CII leader sequence. The results (data not shown) indicate that all three proteins are being expressed and are of the predicted molecular weight. The results show that the absence of reactivity with seropositive sera is not a technical artifact.

In summary, we have systematically cloned the contiguous regions of the HIV-2 envelope protein starting at the amino terminus and covering over 85% of the molecule. The results show that K1 reacts with all HIV-2 positive samples that we have tested. Other regions show little reactivity with these samples. There is no data to correlate the serology with patient health status.

PURIFICATION OF K1 PROTEIN

Fermentation Procedure

E. coli containing the plasmid coding for K1 protein were grown in LB media with 0.1 mg/ml ampicillin at 32°C overnight. The overnight culture was added to a 15 L fermenter with DO and pH control containing LB media supplemented with 1% glucose, 50 mM phosphate and 0.1 mg/ml ampicillin. Fermentation was carried out at 32°C until the culture reached an OD 550 of 1.0-1.1. Expression of K1 was then induced by increasing the temperature to 42°C and the cultures maintained under these conditions for 2 hours. At the end of the induction, cells were harvested by centrifugation at 3,000 x g for 30 minutes. The pellets were frozen at -70°C until use.

K1 protein is found in insoluble inclusion bodies within the host cell, representing 5-10% of total E. coli protein content. E. coli cells were suspended in 50 mM Tris HCl, pH 7.5, containing 2mM PMSF, 50 ug/ml aprotinin and lysed with lysozyme to release inclusion bodies. The inclusion bodies were then subjected to a series of washings that included two washes with

10% Triton X-100, and one wash each with 0.5% Zwittergent 3-14, 1 M NaCl, 4M urea and 8M urea. The inclusion body preparation was then solubilized with 6M guanidine HCl, 50mM Tris HCl, pH 10.0, 0.5% beta-mercaptoethanol. The soluble fraction, obtained by centrifugation at 23,000 X g for 30 minutes was adjusted to pH 8.5 and alkylated with iodoacetic acid at 1.1 molar excess over beta-mercaptoethanol. After alkylation, the sample was dialyzed against 50mM borate, pH 9.0 to remove urea. After dialysis the K1 protein precipitates. The K1 precipitates were redissolved in 8M urea, 50mM borate, pH 9.0, and acylated with citraconic anhydride at 50-fold molar excess over the number of lysyl residues on the K1 protein.

The protein was then dialyzed against the same buffer with no urea, remaining soluble in the absence of denaturant. After dialysis, the K1 protein was purified on a DEAE-TSK column eluted with 0.1 M NaCl gradient. The citraconylated K1 protein was then deacylated under acidic conditions. The purified protein, at 0.4 mg/ml in 50 mM sodium borate, pH 9.0 was adjusted to pH 5.0 with 1.5 M sodium citrate and incubated at room temperature. Deacylation of K1 protein was complete after 20 hours of incubation.

Figure 6 shows a copy of an acrylamide gel of the purified K1 protein. K1 protein migrated at approximately 36 KD. Minor bands migrating faster and slower than the 36 KD major protein were observed. These minor proteins were related to the K1 protein because of their immunoreactivity with sera from HIV-2

seropositive individuals and reactivity with rabbit antisera raised against K1 protein. These bands do not react with rabbit sera raised against E. coli protein (data not shown).

The purified K1 protein was then tested by Western blotting for reactivity with sera from HIV-2 seropositive individuals. As seen with previous Western blot strips, the K1 protein identified all samples from HIV-2 seropositive individuals (data not shown). This further supports the conclusion that K1 represents the immunodominant region of the HIV-2 envelope protein. The purified K1 protein was then used to develop an HIV-2 EIA.

HIV-2 EIA

The experiments outlined above support the conclusion that K1 represents the immunodominant region of the HIV-2 envelope protein. The purified K1 protein was used to develop an HIV-2 EIA.

K1 antigen was diluted in 50 mM sodium bicarbonate, pH 9.6, to a concentration of 0.5 ug/ml. 200 uL aliquots were added to each well of a 96 well polystyrene plate. After incubation at room temperature for 24 hours, excess antigen was removed and the wells were blocked at room temperature with 200 uL of a 4% BSA solution prepared in 0.1 M sodium citrate, pH 5.0. After blocking, plates were washed with PBS to remove residual citrate buffer.

EIA's were performed by incubating antigen coated wells with 200 uL of test sera prediluted 1:20 in sample diluent. Samples were incubated at 37°C for 1 hour. After incubation, wells were

washed 6 times with 0.05% detergent in distilled water. A 200 uL volume of HRP conjugated goat anti-human IgG peroxidase (HRP) was then added. Plates were incubated at 37°C for 30 minutes, followed by 6 washes with the same washing solution. After washing, 200 uL of TMB substrate was added and color was allowed to develop in each well for 15 minutes at room temperature. The reaction was stopped by the addition of 100 uL of 1 M sulfuric acid. OD 450 was read in a microtiter plate reader.

Figure 7 shows the distribution of ELISA signals of HIV-2 positives (71 tested) and negatives (278 tested) using the K1 protein on plates. There is a good distribution between the positives and negatives among the samples tested. We have compared our HIV-2 EIA results with viral lysate Western blots using commercially available kits. The results shown in Table 2 show the correlation between EIA and Western blot.

Optimization of HIV-2 EIA Comparison of Monoclonal vs. Polyclonal Antibody Conjugates

Initial results were based on EIAs utilizing goat polyclonal anti-human IgG labeled with HRP. For technical reasons (see below) EIAs are being converted to using HRP conjugated anti-human monoclonal antibodies to replace the goat polyclonal conjugate. The monoclonals were generated by immunizing Balb/c mice with either human IgM or IgG Fc. Spleens from immunized mice were fused with SP2/0 cells and hybrids screened on the immunizing Fc protein. We have tested the anti-human IgG and IgM monoclonals in EIAs using K1 protein. The results are shown in

Figure 8. The data indicate that the monoclonal conjugates show less background than the polyclonal antibody for the negative samples that we have tested. There is a negligible loss of signal from the seropositive samples. Our conclusion, based on these results, is that the monoclonal conjugate is superior to the polyclonal because of (a) the lower background with negative samples due to a decrease in non-specific binding, (b) the absence of effect on seropositive samples, and (c) increased uniformity in conjugate preparation. The tests that we have provided utilize the monoclonal conjugates.

Other Optimizations

During the course of optimization, we have tested:

Antigen Concentration - Antigen was titered on the plates in the range of 0.1 to 1.0 ug/ml. Optimum titration, based on positive to negative signal ratio, was found to be 0.5 ug/ml equivalent to 100 ng/well.

Stability of Kit Reagents - Stability testing of the kit is ongoing. To date we have data on plates and conjugate for 6 months at 4°C. All other reagents have a two year shelf life.

Interfering Factors - Samples from individuals that have tested positive for hepatitis, herpes, CMV, RF and other interfering agents, but negative for HIV-1, have been tested in the HIV-2 assay and no interference has been found.

Conjugate Validation - Monoclonal antibodies were conjugated three separate times and all three lots were validated in the HIV-2 assay using a series of positive and negative samples.

Assay Kinetics - Kinetics were tested by varying incubation times and temperatures for all incubation steps. Ratios of positive to negative samples were evaluated to ensure that equilibrium has been reached at all steps.

Our HIV-2 EIA has been optimized for all of the above parameters.

Reactivity of Samples from HIV-2 Seropositive Individuals in an HIV-1 env & gag assay.

We have tested HIV-2 samples from Africa on our HIV-1 env & gag EIA to determine the percentage of samples that cross reacted. Out of 55 samples, 40 (72%) were positive using the cutoff calculation of Positive Control X 0.4 for the env & gag assay. If the cutoff was decreased 20% (to approximately 0.32 X Positive Control), the number of samples reading positive goes to 43 (78.2%). In the complementary experiment, we tested HIV-1 seropositives in the HIV-2 EIA. Of the 54 samples tested, 15 (28%) were positive while the remainder were negative (38, 72%).

SUMMARY

Using recombinant DNA techniques, we expressed in E. coli a protein called K1 that contains an immunodominant epitope of the HIV-2 envelope protein. The location of K1 within HIV-2 is similar to that of CBre3, a protein derived from the HIV-1 envelope. CBre3 has been cloned and expressed, and its utility as a diagnostic for HIV-1 infection has been established (11).

In this report, we show that K1 serves a similar role for HIV-2. K1 has been expressed at high levels and techniques

developed for large scale purification. The purified protein has been used in an EIA to identify HIV-2 seropositive samples of West Africa origin. While only a limited number of HIV-2 seropositive samples have been tested (approximately 80), the HIV-2 EIA using K1 has high specificity and sensitivity.

Finally, we have expressed other regions of the HIV-2 envelope gene and tested for reactivity with sera from HIV-2 infected individuals. Results show that these other coding regions are poorly reactive. We have no information to correlate reactivity with health status of infected individuals.

REFERENCES

1. Clavel, F., Guetard, D., Brun-Vezinet, F., Chamaret, S., Rey, M-A, Santos-Ferreira, Mo., Laurent, A.G., Dauguet, C., Katlama, C., Rouzioux, C., Klatzmann, D., Champalimaud, J.L., and Montagnier, L. (1986) Science, 233:343-346.
2. Guyader, M., Emerman, M., Sonigo, P., Clavel, F., Montagnier, L., and Alizon, N. (1987) Nature, 326:662-669.
3. Zagury, J.F., Franchini, G., et al., (1988) Proc. Natl. Acad. Sci., (USA) 85:5941-5945.
4. Horsburgh, C.R., Jr. and Holmberg, S.D. (1988) Transfusion, 28:192-195.
5. Cortes, E., Detels, R., et al. (1989) N. Engl. N. Med., 320:953-959.
6. Brun-Vezinet, F., Katlama, C., et al. (1987) Lancet, 1:128-132.
7. Clavel, F., Mansinho, K. et al. (1987) N. Engl. J. Med., 316:1180-1185.
8. Franchini, G., Fargnoli, K.A., et al. (1989) Proc. Natl. Acad. Sci. (USA) 86:2433-2437.
9. Lautenberger, et al. (1984) Gene Anal. Tech., 1:63.
10. Sambrook, J., Fritsch, E.F., and Maniatis, T. (1989) Molecular Cloning, Cold Spring Harbor Laboratory Press.
11. Marciani, D.J., Thorn, R.M., Riggan, C.H, Beltz, G.A., and Hung, C-H, (1989) Recent Advances in Pharmacology and Therapeutics. Valesco et al. eds.
12. Thorn, et al. (1987) J. Clin. Microb., 25:1207-1212.

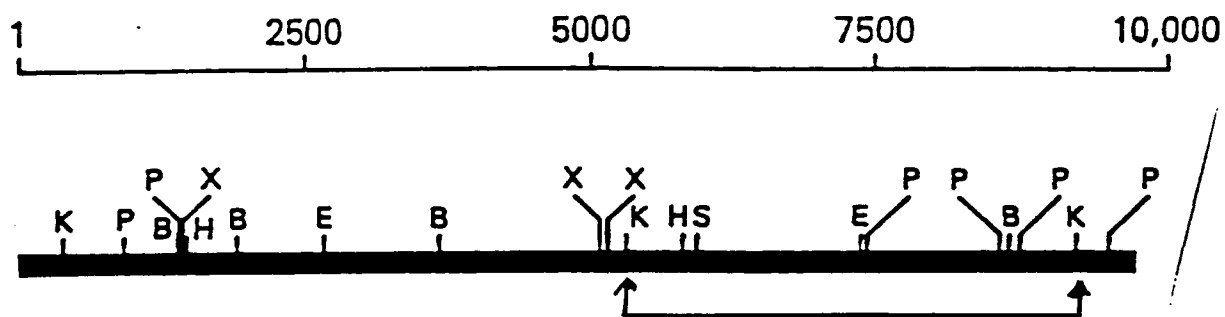


FIGURE 1. Location of the K3D DNA fragment within the HIV-2_{SBL/ISY} clone. K3D is a 2.7 KB KPN I fragment containing the entire envelope gene and flanking viral DNA. K3D is derived from the cloned virus HIV-2_{SBL/ISY} and was kindly provided by Dr. G. Franchini.



FIGURE 2. Hydrophobicity map of HIV-2 envelope protein. Subfragments expressed are depicted below.

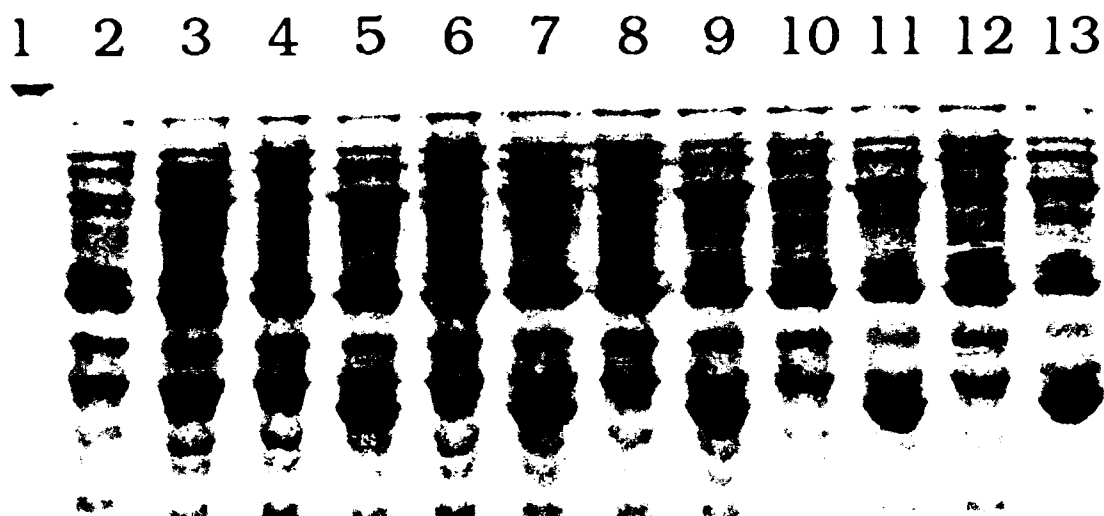


FIGURE 3. Coomassie stained acrylamide gel of proteins from uninduced and induced cultures of HIV-2 recombinants. Lanes 5,7,9,11,13 show the induced K1 protein of HIV-2 (arrow).

1 2 3 4 5 6 7 8 9 10 11 12 13

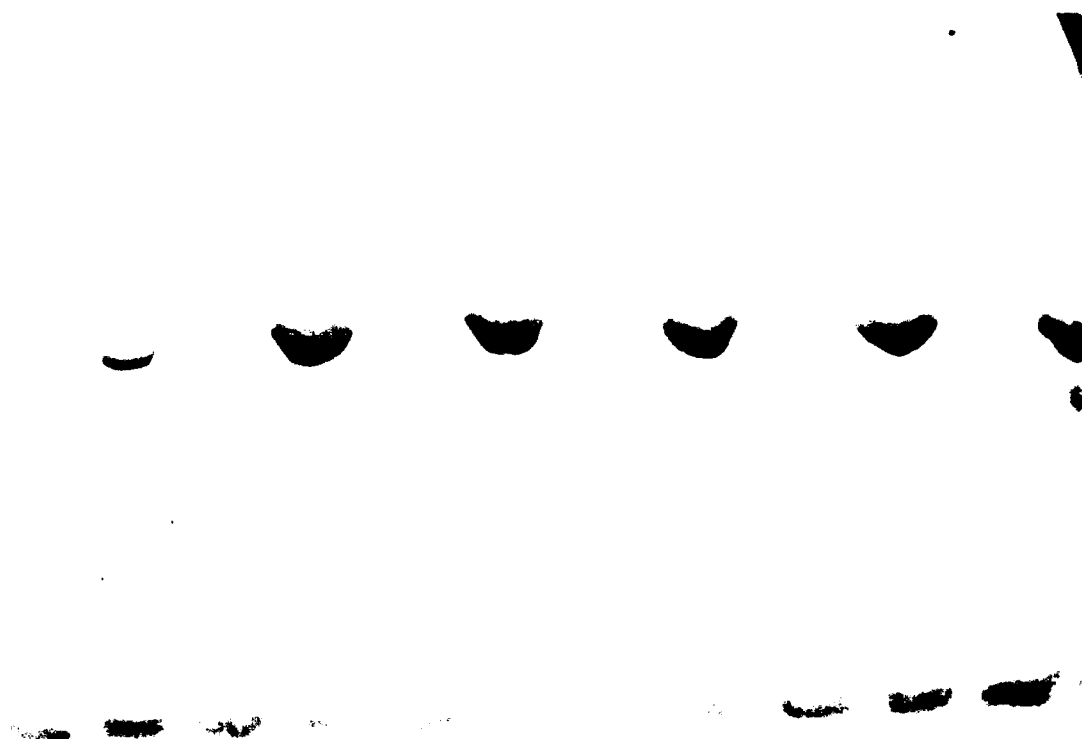


FIGURE 4. Western blot of gel depicted in Figure 2. Blot is reacted with antibody raised against the crossreacting ACC1 protein of SIV.

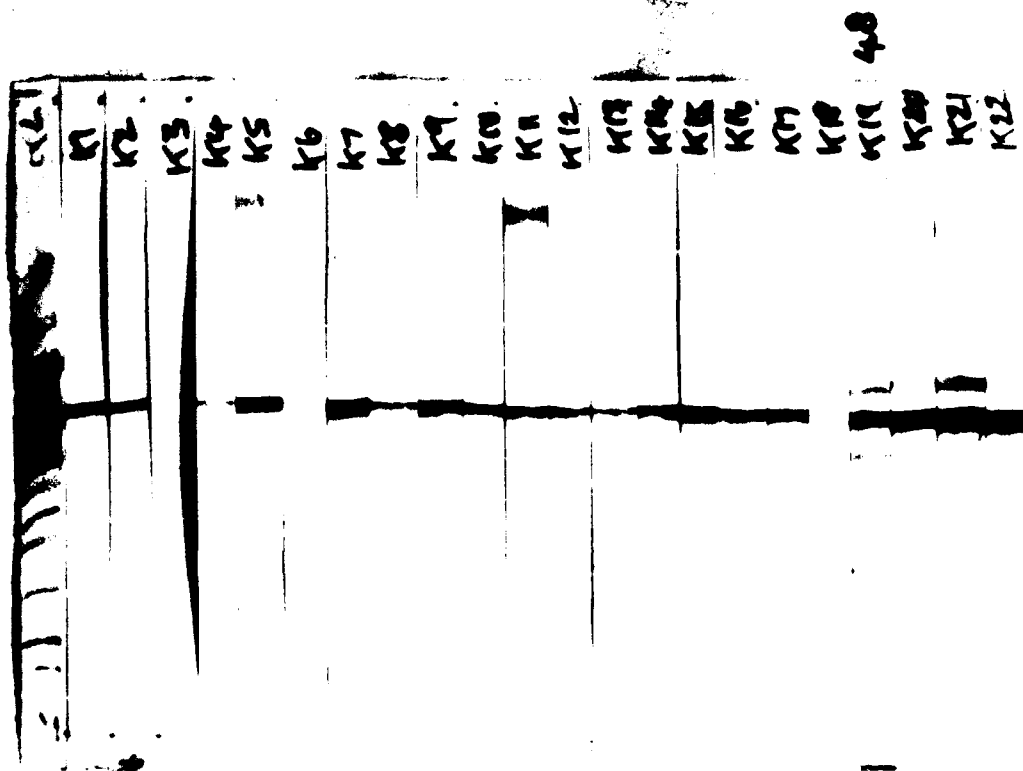


FIGURE 5. Strip Western blots of K1 protein reacted with presumed HIV-2 positive sera. See text.

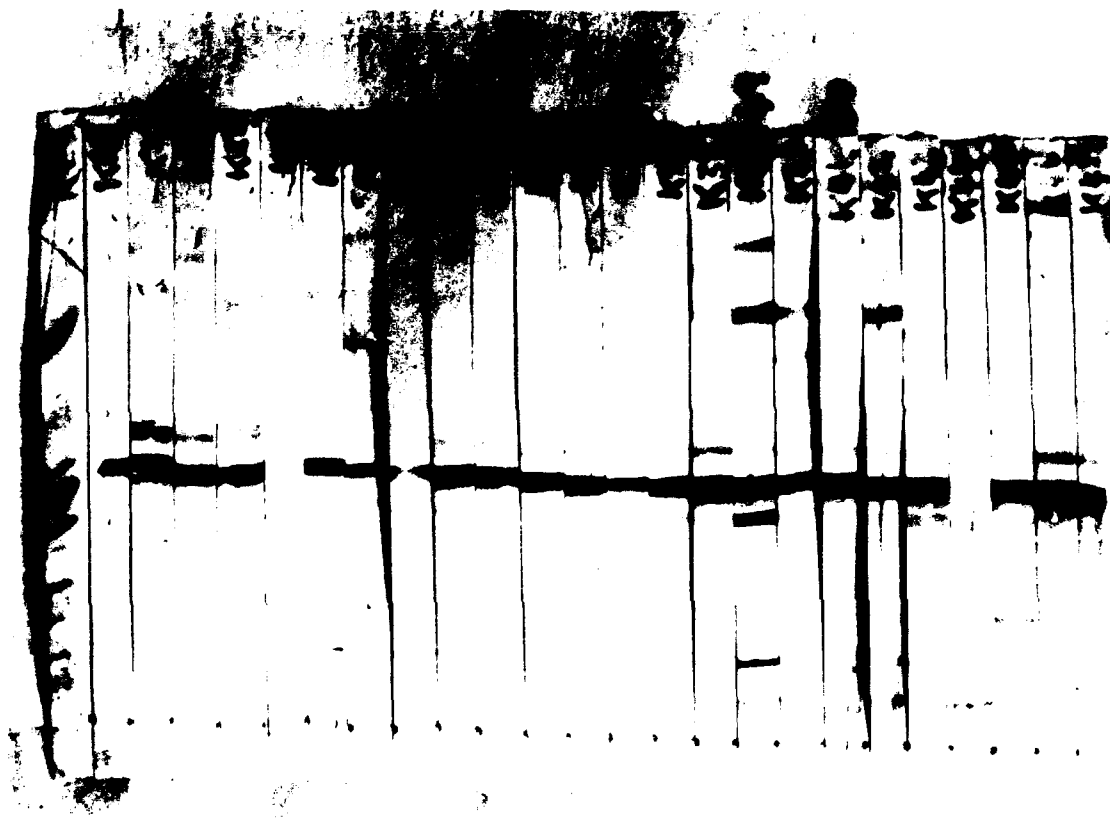


FIGURE 5. (continued)

TABLE I

REACTIVITY OF RECOMBINANT HIV-2 PROTEINS

CONSTRUCT	NO. TESTED	POSITIVE
K18	23	4
K13	28	0
K2	18	2

TABLE II

**COMPARISON OF RECOMBINANT HIV-2 env
AND HIV-2 VIRAL LYSATE WESTERN BLOTS**

	Viral Lysate Western Blot	
	Pos.	Neg.
HIV-2 <u>env</u> EIA		
Pos.	46	0
Neg.	0	24

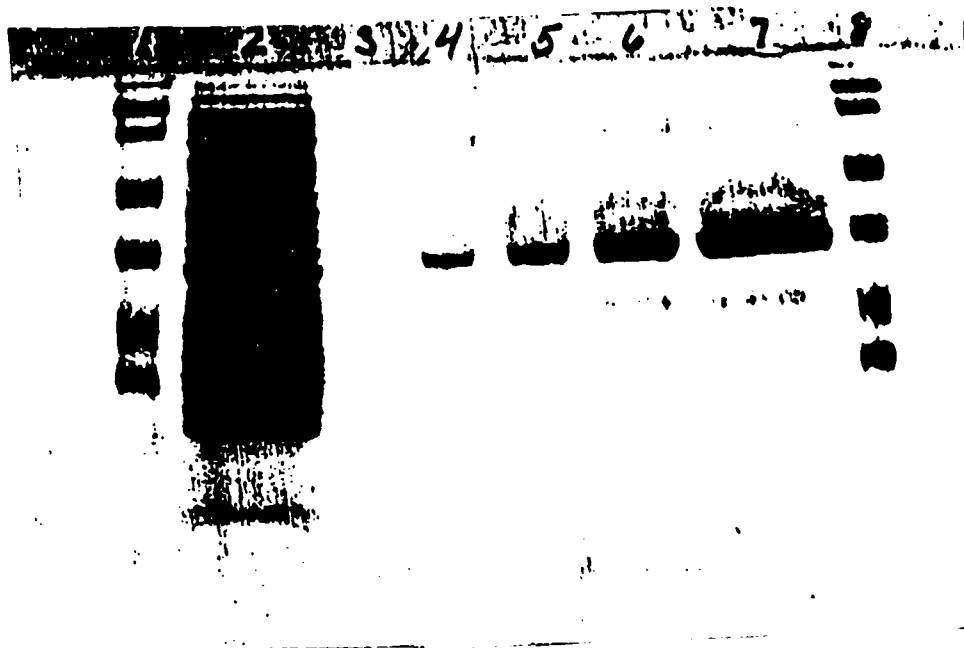


Figure 6. Coomassie Stained Acrylamide Gel of Purified K1 Protein

Lane 1	Molecular Weight Standard
Lane 2	Whole E.coli lysate
Lane 3	Blank
Lane 4	0.4 ug Purified K1
Lane 5	1.0 " " "
Lane 6	2.0 " " "
Lane 7	4.0 " " "
Lane 8	Molecular Weight Standard

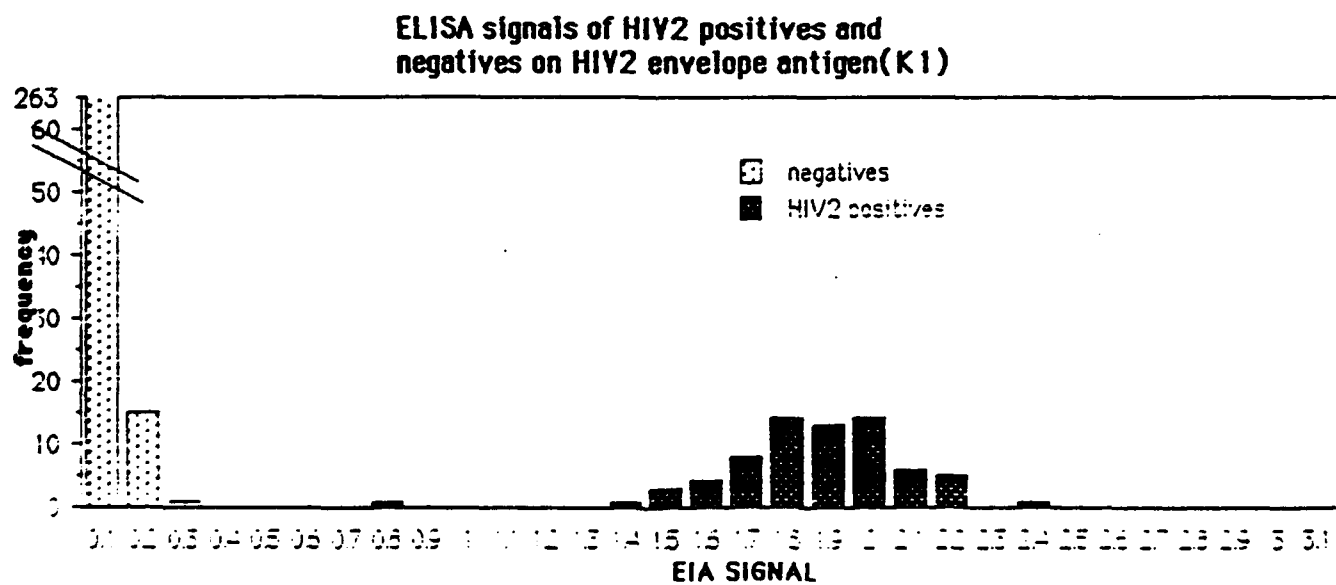
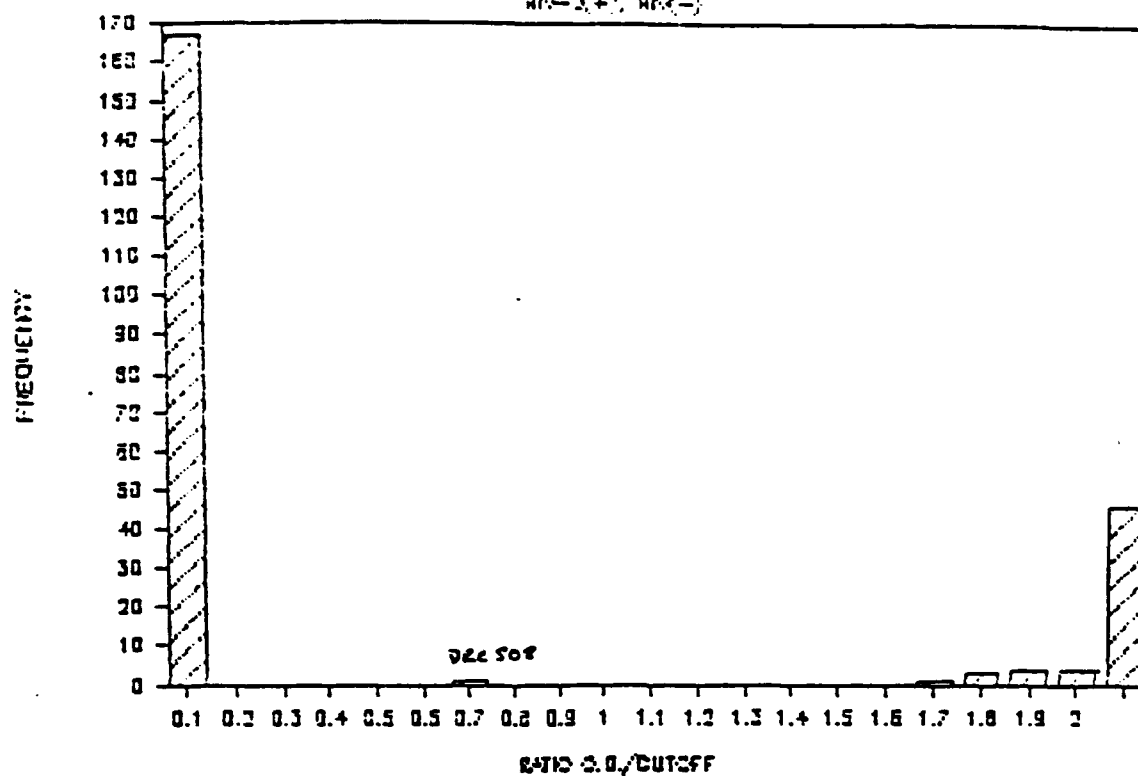


FIGURE 7

MONOCLONAL AB CONJUGATE

HP(-)(+), HP(-)(-)



POLYCLONAL AB CONJUGATE

HP(-)(+), HP(-)(-)

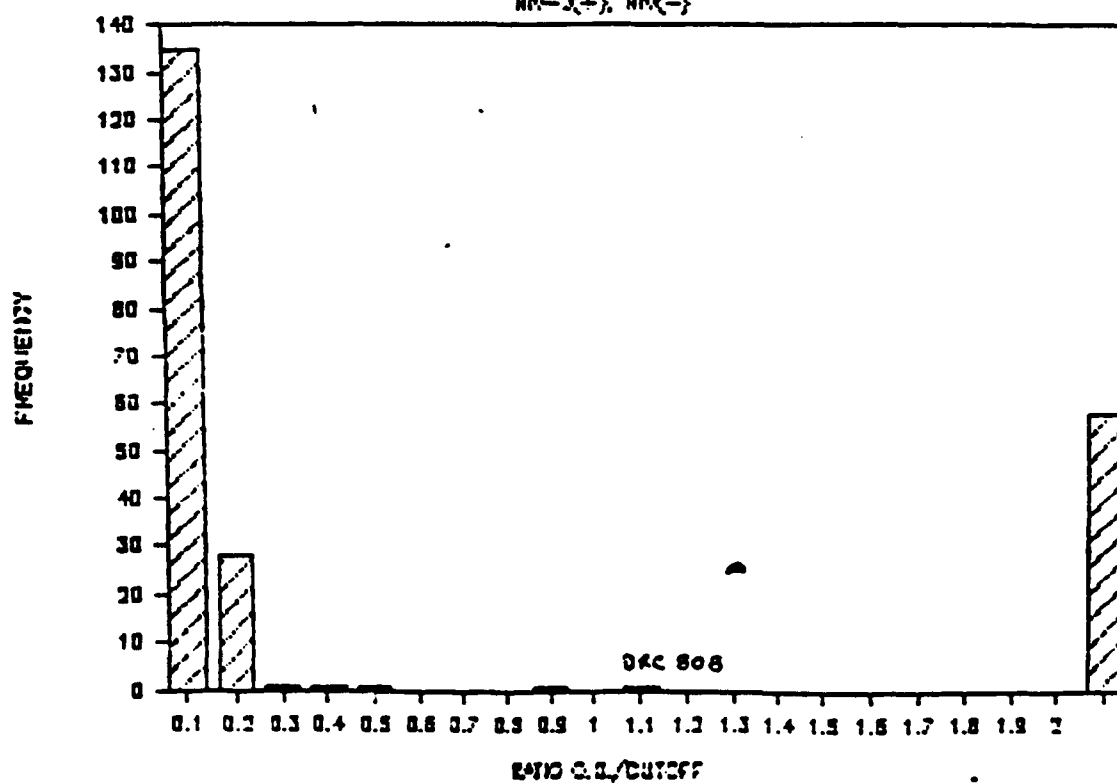


FIGURE 8